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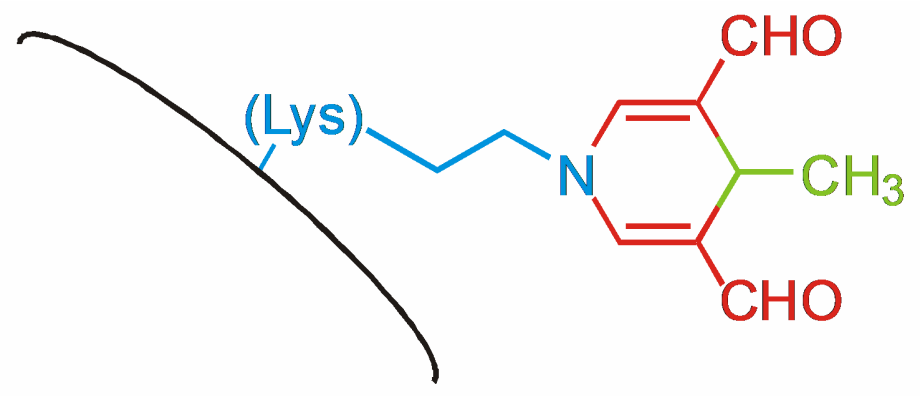
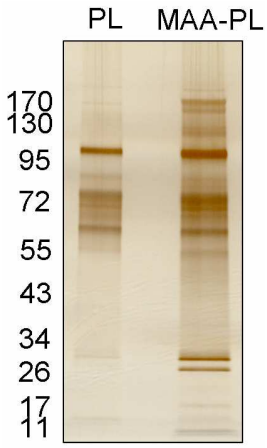
SUPPLEMENTARY FIGURES



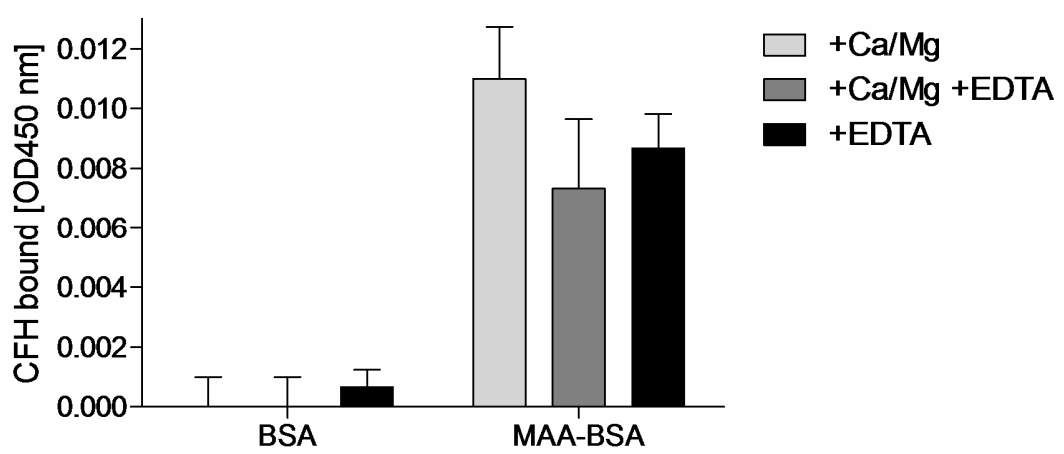
Supplementary Fig. 1: Example of an advanced MDA-adduct

MAA (malondialdehyde-acetaldehyde adduct) is a specific and prominent advanced MDA adduct formed by the condensation of two MDA (red) and one acetaldehyde (derived from breakdown of MDA, green) molecules reacting with the ε-amino group of lysine (blue). All proteins and lipids containing primary amine groups can potentially be modified by MAA.



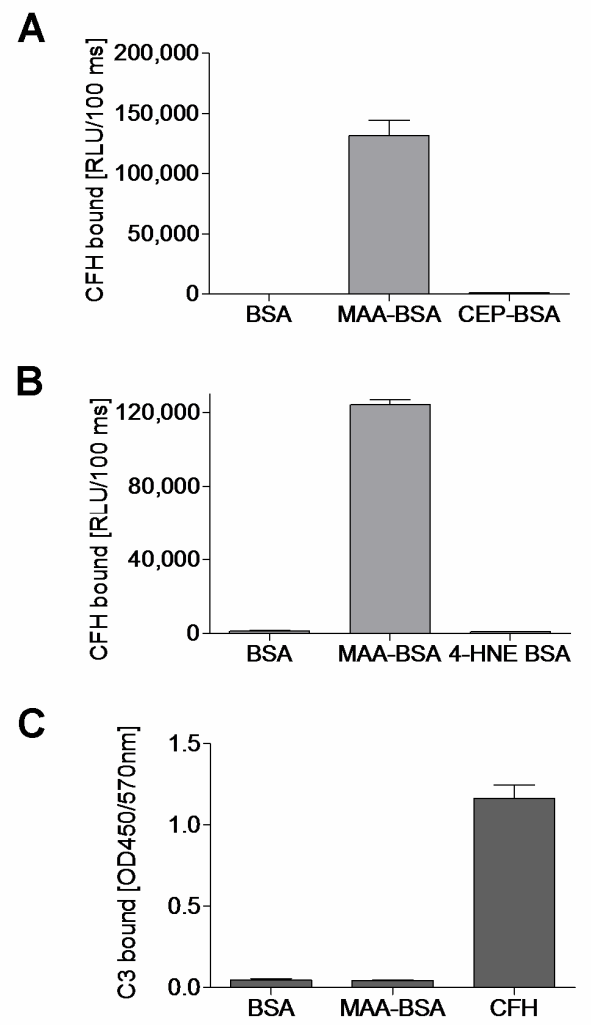
Supplementary Fig. 2: Proteomic identification of CFH

Eluates from either polylysine (PL) or MDA-polylysine (MDA-PL) beads incubated with plasma from $RAG^{-/-}LDLR^{-/-}$ mice were separated by one-dimensional SDS-PAGE and the proteins were stained with silver nitrate. Twenty regions were excised from each lane, digested *in situ* with trypsin and analysed by LC-MSMS.



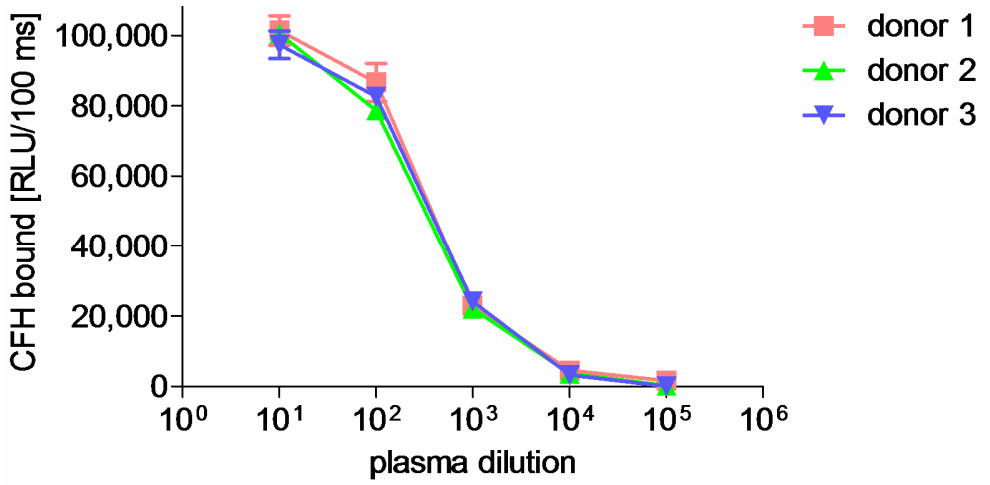
Supplementary Fig. 3: Interaction of CFH with MDA is independent of divalent cations

ELISA showing the binding of the CFH to coated BSA and MAA-BSA in the presence of $MgCl_2$ (1mM), $CaCl_2$ (2mM) and disodium EDTA (10mM). CFH binding was not affected by the addition of the divalent cation-chelator EDTA. Values are given as OD (450/540nm) and represent the mean±SD of triplicate determinations.



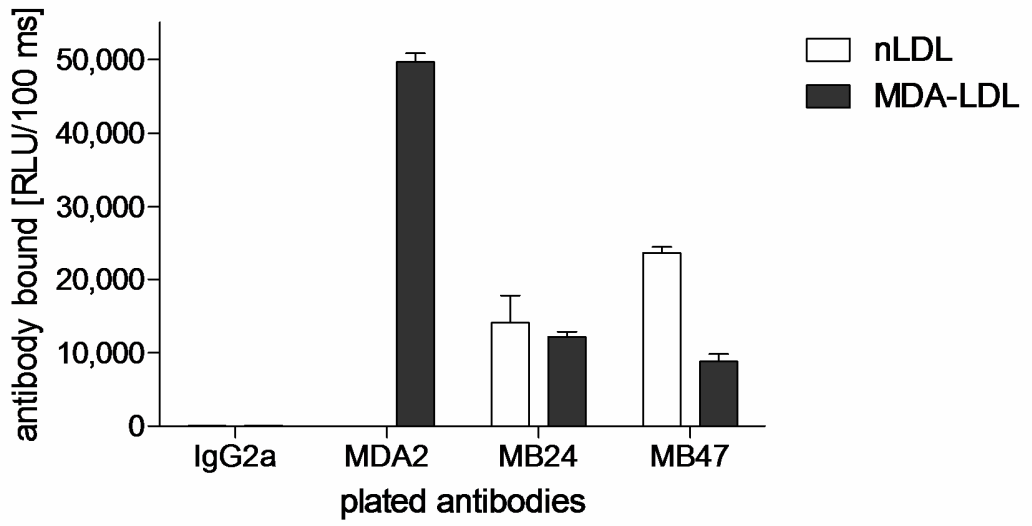
Supplementary Fig. 4: Interaction of CFH with MAA is specific

(A-B) CFH does not bind to CEP or 4-HNE. ELISA for CFH binding. Shown is the binding of purified CFH (2µg/ml) to coated BSA, MAA-BSA and CEP-BSA **(A)** or 4-HNE-BSA **(B)** as determined by chemiluminescent ELISA. Values are given as relative light units (RLU) per 100ms and represent the mean±SD of triplicate determinations. **(C)** C3 does not bind MDA. ELISA for C3 binding. Shown is the binding of purified C3 (5µg/ml) to coated BSA, MAA-BSA and CFH (a known interactor of C3) as determined by ELISA. Values are given as optical density (OD) at 450/570nm and represent the mean±SD of triplicate determinations.



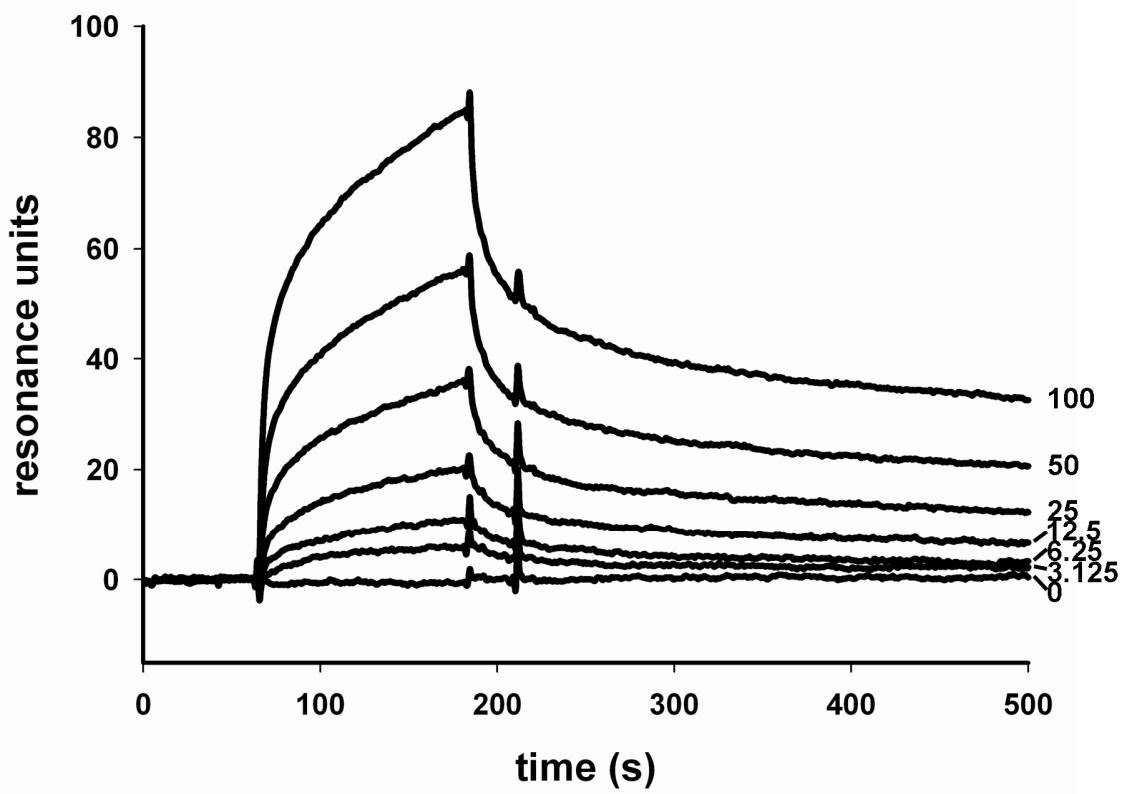
Supplementary Fig. 5: Dose dependent interaction of plasma CFH with immobilized MAA-BSA

ELISA showing the binding of CFH to coated MAA-BSA in different dilutions of plasma donated by three healthy individuals. Values are given as RLU per 100ms and represent the mean±SD of triplicate determinations.



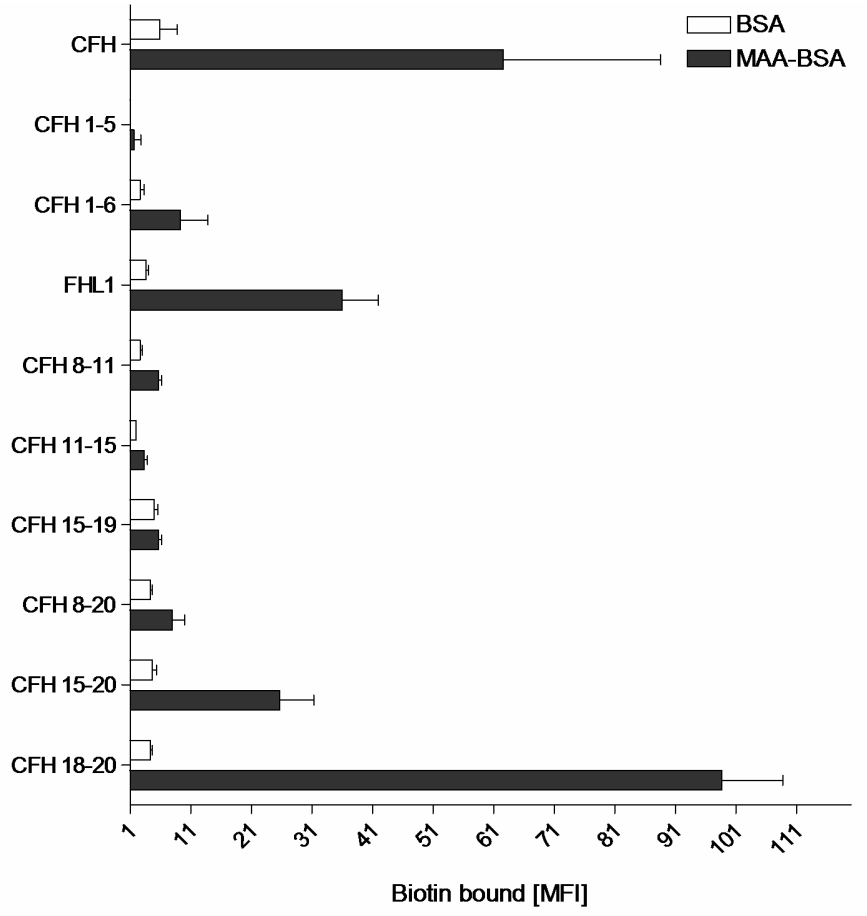
Supplementary Fig. 6: Antibody binding to native and MDA-modified LDL

Chemiluminescent immunoassay showing the binding of the MDA2-, MB24-, and MB47 mAbs and an IgG2a isotype control mAb to coated native LDL and MDA-LDL. Bound antibody was detected with alkaline phosphatase conjugated anti-mouse-IgG. Data are the mean of triplicate determinations.



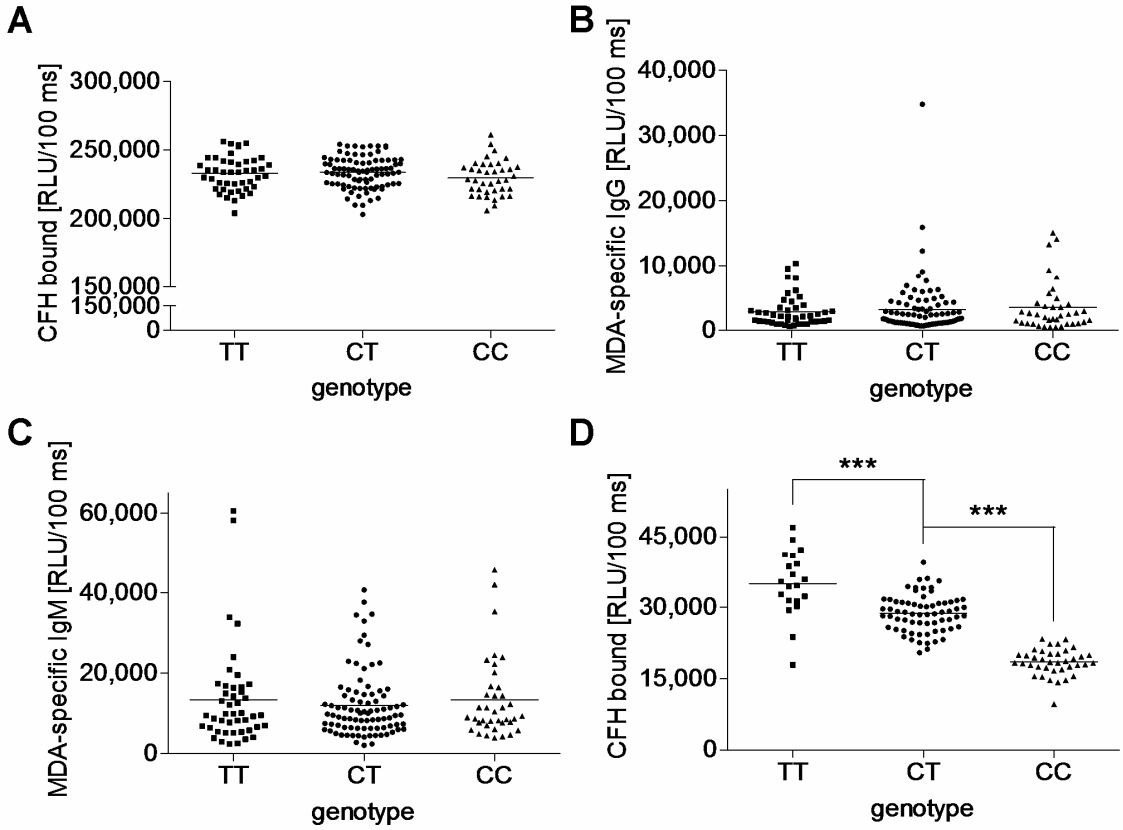
Supplementary Fig. 7: Dose dependent interaction of purified CFH with immobilized MAA-BSA

MAA-BSA was coupled to the flow cell of a CM5 sensor chip. Purified CFH was applied in fluid phase with the indicated concentrations.



Supplementary Fig. 8: Binding of biotinylated MAA-BSA to coated CFH fragments

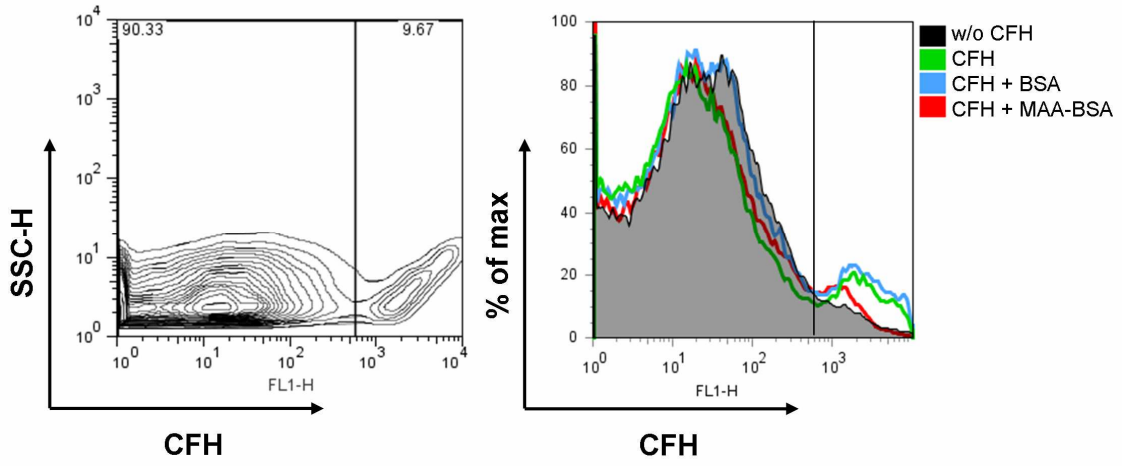
Binding of biotinylated BSA (white bars) or MAA-BSA (black bars) to coated CFH fragments (5µg/ml) as determined by chemiluminescent ELISA. Values are given as MFI (mean fold increase of chemiluminescence above background) and represent the mean±SD of triplicate determinations.



Supplementary Fig. 9: AMD patient plasma characterization

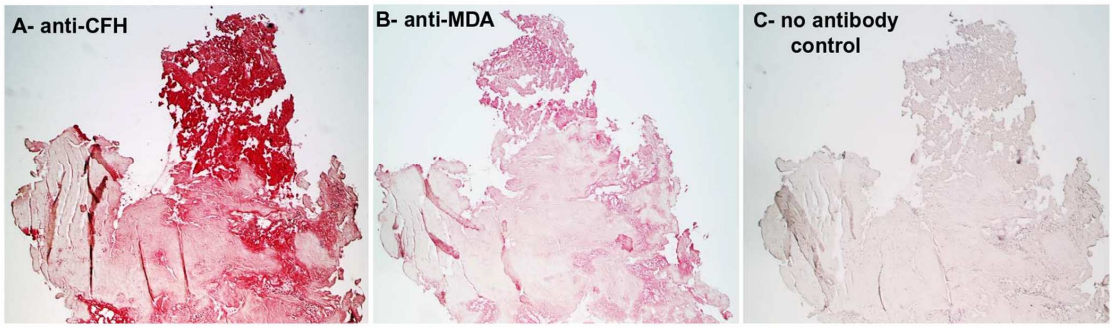
(A) CFH levels in patient plasma. ELISA showing the total amount of CFH in subject plasma according to genotype. Plasma of subjects homozygous for the 402H allele (CC, n=38), heterozygous for the 402H allele (CT, n=88) or homozygous for the wildtype 402Y allele (TT, n=45) was diluted 1:250 and added to microtiter wells coated with guinea pig anti-CFH. The relative amount of CFH binding was then determined by chemiluminescent ELISA using the biotinylated guinea pig anti-mouse-CFH antibody. Symbols represent individual subject samples with horizontal bars indicating the mean of each group. Values are given as RLU per 100ms and represent the mean of triplicate determinations. **(B-C)** Levels of MDA-specific IgG and IgM. ELISA for binding of IgG **(B)** and IgM **(C)** in subject plasma according to genotype. Plasma was diluted 1:250 and added to microtiter wells coated with MDA-LDL. The relative amount of IgG or IgM binding was then determined by chemiluminescent ELISA using secondary anti-IgG or

anti-IgM antibodies. There was no association with the binding of IgG or IgM to MDA-LDL related to the H402 (C) allele. Symbols represent individual subject samples with horizontal bars indicating the mean of each group. Values are given as RLU per 100ms and represent the mean of triplicate determinations. **(D)** CFH binding without CFHR1/CFHR3 deletions. ELISA for binding of plasma CFH in subject plasma according to genotype. Subjects carrying CFHR1 or CFHR3 deletions were excluded. Plasma of subjects homozygous for the H402 allele (CC, n=38), heterozygous for the H402 allele (CT, n=67) or homozygous for the wildtype y402 allele (TT, n=20) was diluted 1:250 and added to microtiter wells coated with MDA-LDL. The relative amount of CFH binding was then determined by chemiluminescent ELISA using the biotinylated guinea pig anti-mouse-CFH antibody. There was a significant gene-dosage dependent reduction in binding of CFH to MDA-LDL related to the H402 (C) allele. Symbols represent individual subject samples with horizontal bars indicating the mean of each group. Values are given as RLU per 100ms and represent the mean of triplicate determinations. (*** $p < 0.001$).

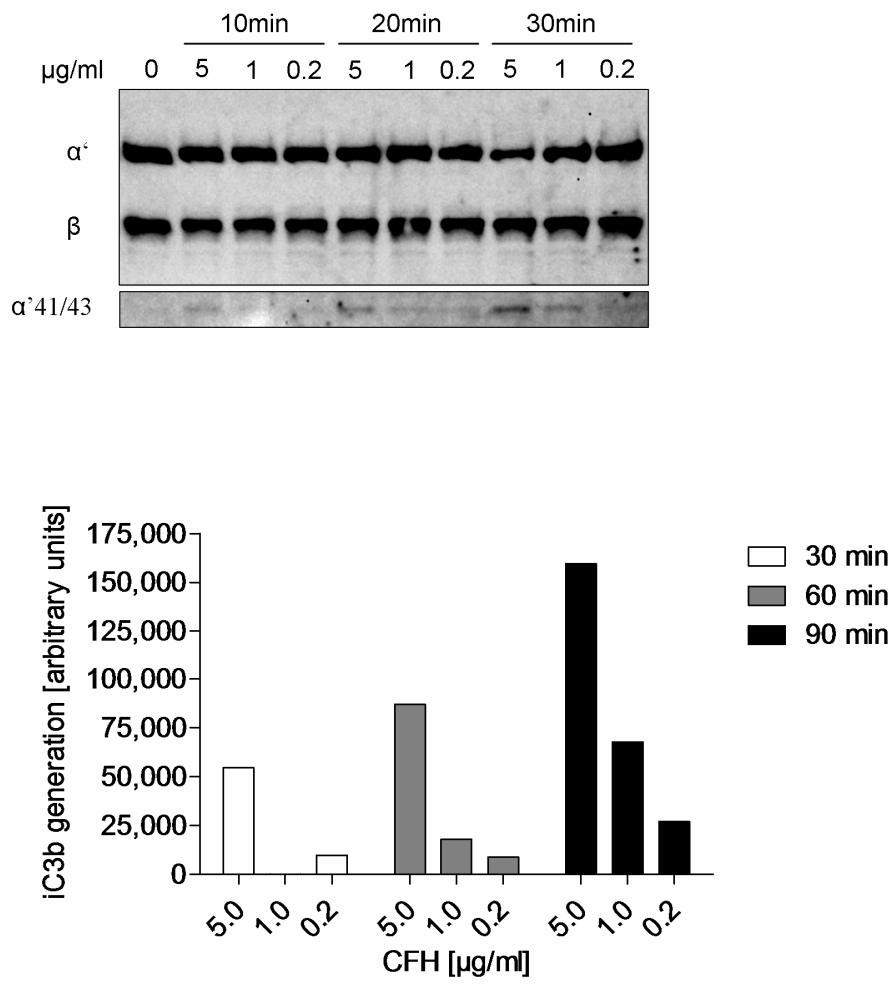


Supplementary Fig. 10: MAA-BSA competes for the binding of CFH to apoptotic blebs

Competition assay for the binding of CFH to apoptotic blebs. Shown is a representative FACS plot of the binding and competition experiment shown in Fig. 3K. The upper panel indicates the percentage of apoptotic blebs bound by CFH. The lower panel represents a histogram showing CFH binding to apoptotic blebs in the absence and presence of BSA and MAA-BSA, respectively. CFH-binding is indicated by FITC-fluorescence on the abscissa.

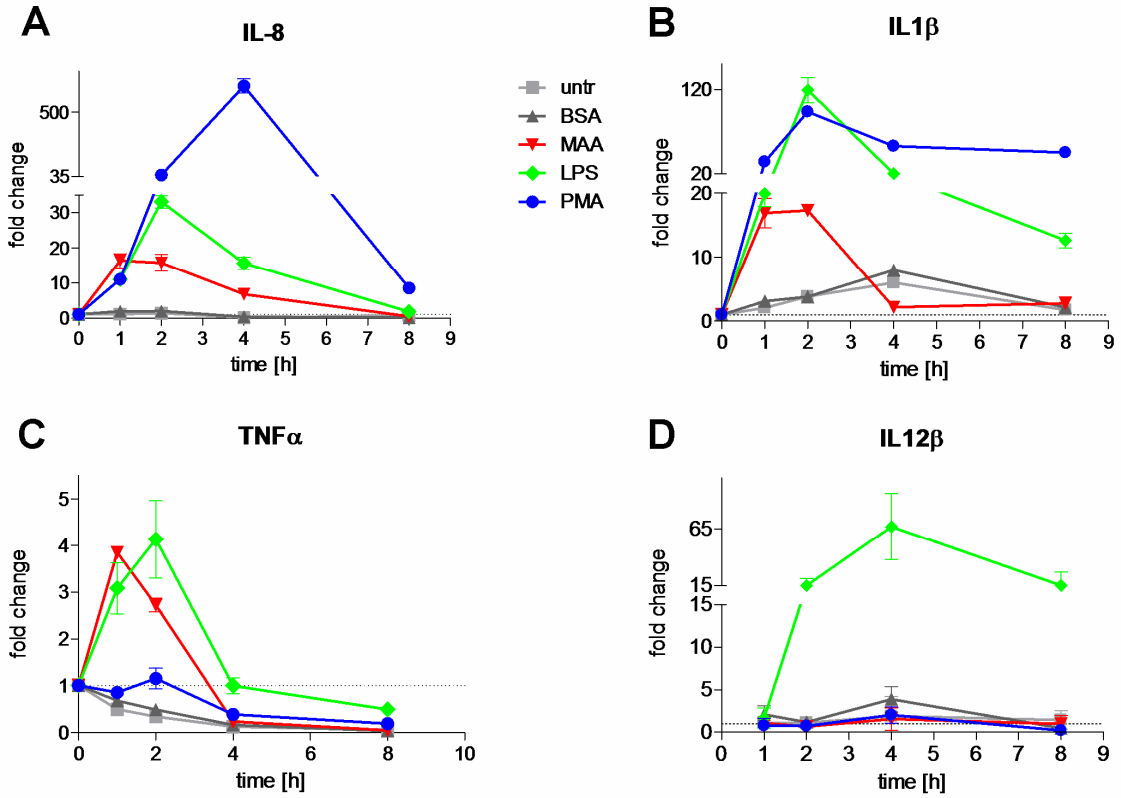


Supplementary Fig. 11: CFH and MDA are present in human atherosclerotic lesion
Shown are immunostained sections of atherosclerotic tissue obtained from a coronary artery at time of therapeutic percutaneous intervention. Parallel sections stained for the presence of CFH with a guinea pig antiserum to CFH (A) and for MDA epitopes with MDA2 (B) and with secondary antibody only as control (C). Positive staining is indicated by the red colour. The morphology is reflective of friable tissue but does show colocalization in large areas of these freshly obtained sections.



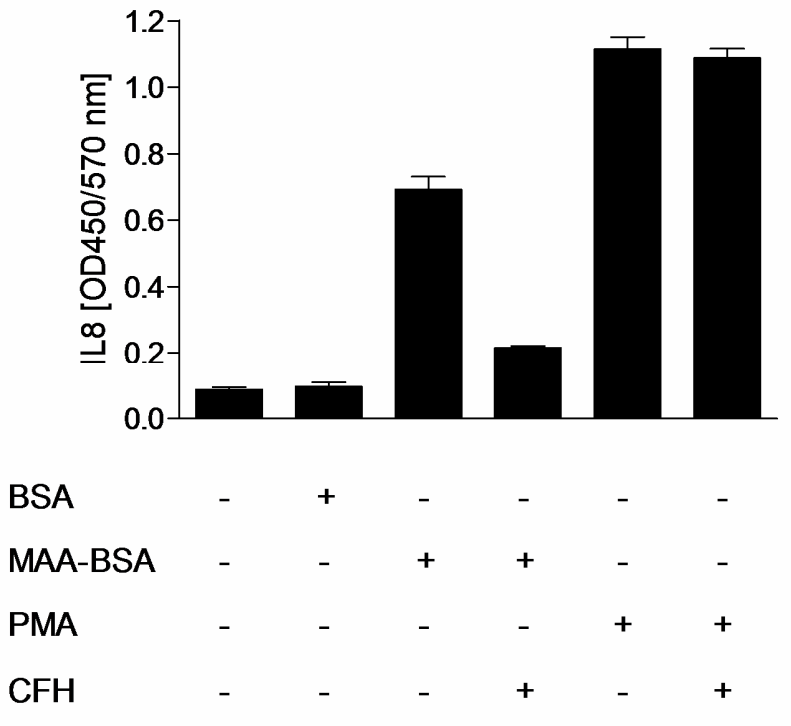
Supplementary Fig. 12: Cofactor activity of CFH bound to MAA-BSA.

CFH bound to coated MAA-BSA was incubated with C3b and factor I for the indicated times and the generation of C3b degradation products was visualized by immunoblotting (upper panel). The 43kDa degradation product iC3b was densitometrically quantified. The results are presented as a bar graph in the lower panel.



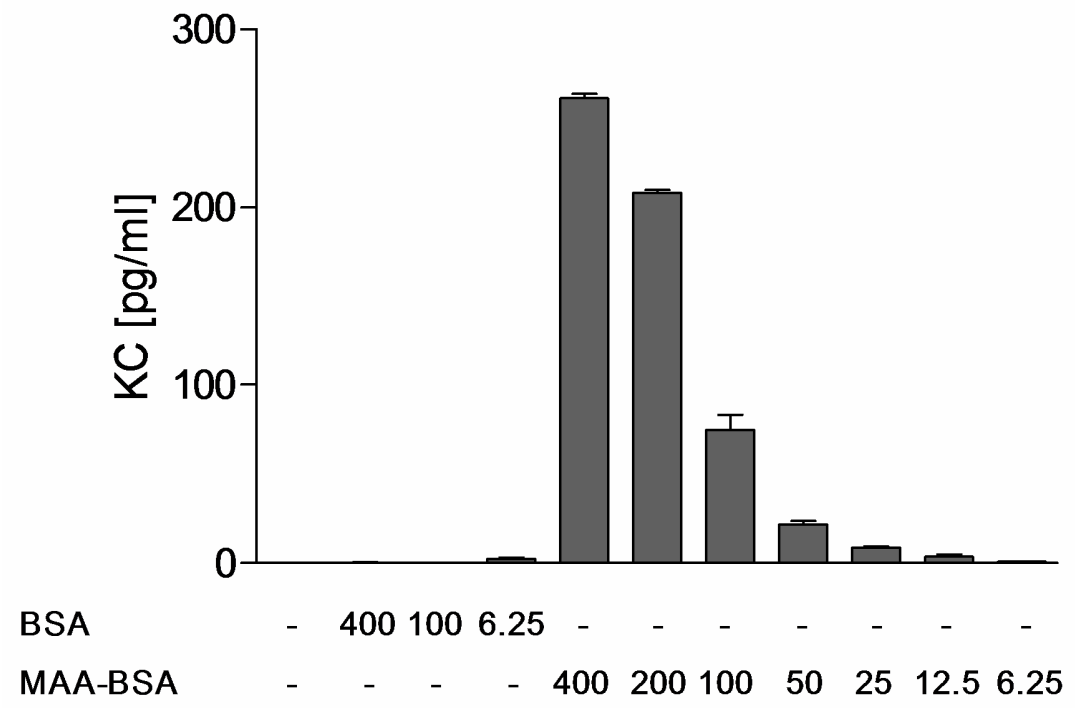
Supplementary Fig. 13: MAA-BSA induces an immediate inflammatory response in THP1 cells

THP1 cells were stimulated with BSA, MAA-BSA, LPS or PMA. After the indicated times, expression of IL12β, IL1β, TNFα and IL8 was determined by RT-qPCR. Data points represent mean±SD of biological triplicates.



Supplementary Fig. 14: CFH does not inhibit PMA-induced IL-8 production

THP1 cells were cultured in the presence of BSA, MAA-BSA, CFH and/or PMA for 16h and supernatants were assayed for the presence of IL-8 by ELISA. Bars represent mean±SD of triplicate determinations.



Supplementary Fig. 15: MAA-BSA induces KC response in mouse bone-marrow derived macrophages

Mouse bone marrow derived macrophages were cultured in the presence of indicated concentrations of BSA or MAA-BSA for 16h and supernatants were assayed for the presence of KC by ELISA. Numbers below indicate concentrations of BSA and MAA-BSA in µg/ml. Bars represent mean±SD of triplicate determinations.

SUPPLEMENTARY METHODS

Subjects and Clinical Diagnosis:

112 patients with a clinical diagnosis of AMD and 67 control subjects of similar age, gender and smoking habits, who showed no signs of macular disease were studied [41]. Mean age of the AMD patient cohort was 75.6 years (SD, 6.6; range, 59-94) and that of the controls was 70.1 years (SD, 6.0; range, 60-86). Within the patient and control cohort, 53% and 52%, respectively, were non-smokers; 38% and 39%, respectively, were ex-smokers; and 9% and 9%, respectively, were current smokers.

Within the AMD cohort, 78 (70%) had choroidal neovascularization (CNV), 25 (22%) had geographic atrophy (GA), and 9 (8%) had early AMD. Digital fundus photographs were obtained from all participants. In patients with CNV, optical coherence tomography and fluorescein angiography were performed. Fundus autofluorescence imaging was performed in patients with GA. All fundus images were graded separately by two independent readers (HPNS and PCI) according to the classification system of the International ARM Epidemiological Study Group [43].

All subjects were of Caucasian descent and were recruited within the same time period. All subjects were characterized by means of a standardized case report form which included smoking history, a comprehensive medical history and current medications. Subjects were excluded if they suffered from diseases known to cause abnormal complement levels. Informed consent was obtained from all subjects.

For immunohistochemistry, globes from 49-93 year old donors were obtained from NDRI (Philadelphia, PA) within 6 hours of death, and were on life support for < 24 hours (Table 1). Macular calottes were fixed for 1 h in 2% paraformaldehyde and then cryoprotected by progressive infiltration in 10% and 20% sucrose in PBS (w/v) before freezing in 2:1 sucrose 20% (w/v):OCT compound at -80 ^{o}C.

Table 1: Patient description for histology specimen

Donor	Age	Gender	D-E (hr)	AMD	Cause of Death	rs1061170
1	49	M	3:45	No	Trauma	tt
2	72	F	6:00	No	Cardiac arrest	ct
3	70	F	6:10	Yes	Lung cancer	ct
4	85	F	2:30	Yes	Multiorgan system failure	tt
5	74	M	2:40	Yes	Brain cancer	ct
6	90	F	3:53	Yes	Congestive heart failure	cc
7	93	F	5:45	Yes	Renal Failure	cc

Atherectomy material was collected during percutaneous coronary intervention through catheter aspiration and immediately fixed in EDTA/BHT/paraformaldehyde. Subsequently, the fixed specimens were embedded in paraffin and sections prepared for immunohistochemistry.

The research protocols were in keeping with the provisions of the Declaration of Helsinki, and approval was obtained from the respective institutional ethics committees (Rheinische Friedrich-Wilhelms University Bonn, Germany; Human Research Protections Program of the University of California San Diego, USA).

Plasma Samples:

Venous blood was collected from all subjects into tubes containing dipotassium EDTA at a final concentration of 8 mM. The plasma was separated from the blood cells by centrifugation (20 min/1,000 x g) within 3 hours after venipuncture and frozen in aliquots at -80°C until analysis. All subjects had normal creatinine and cystatin C values, and the two subject groups were not significantly different for these variables. CFH levels in plasma were measured as previously described [44].

Genotyping:

Genomic DNA was extracted from peripheral blood leukocytes following established protocols. Genotyping was done by TaqMan SNP Genotyping or by direct sequencing of SNPs. TaqMan Pre-Designed SNP Genotyping Assays (Applied Biosystems, Foster City, U.S.A.) were performed according to the manufacturer's instructions and were analyzed with a 7900HT Fast Real-Time PCR System (Applied Biosystems). Direct sequencing was performed with the Big Dye Terminator Cycle Sequencing Kit Version 1.1 (Applied Biosystems) according to the manufacturer's instructions. Reactions were analyzed with an ABI Prism Model 3130xl Sequencer (Applied Biosystems). Individual genotypes that were ambiguous or missing were reanalyzed resulting in a call-rate of 100% for all SNPs tested [4145]. The CFHR3/CFHR1 allele distribution in the studied patient cohort is listed in table 2. Genetic associations with CFH binding to coated MDA-LDL binding were assessed using PLINK version 1.07 [46].

Table 2: Patient genotypes for plasma samples

No. of alleles CFHR3/CFHR1	% of total		
	T/T	C/T	C/C
0/0	6.7	0.0	0.0
0/1	0.0	0.0	0.0
1/0	0.0	1.1	0.0
1/1	44.4	15.9	0.0
1/2	0.0	0.0	0.0
2/1	2.2	3.4	0.0
2/2	44.4	76.1	100.0
undet.	2.2	3.4	0.0

When DNA was extracted from histological specimen, QIAamp FFPE Tissue Kit (Qiagen) was used. Genotyping was performed in the Molecular Diagnostic Laboratory of the Medical University of Vienna using a certified kit.

Intravitreal injection:

C57BL/6 mice were anesthetized and the pupils dilated. Using a dissecting microscope, intravitreous injections into one eye of each mouse (n=5 per group) were performed with a pump microinjection apparatus (Harvard Apparatus, Holliston, MA) and a glass micropipette that was calibrated to deliver 1ul of vehicle containing either BSA (2.34 ug), MAA-BSA (2.34 ug), CFH (2.2ug), or a combination of CFH and BSA-MAA(4.4ug of CFH and 2.4 ug of BSA-MAA) pre-incubated for 1 hour, on depression of the foot switch. Six hours later, mice were sacrificed, eyes were enucleated, and the RPE/choroid was dissected.

Total RNA was extracted from RPE/choroid and retina using the Qiagen RNeasy mini kit (Qiagen, Valencia,CA), reversely transcribed using the high capacity RNA to cDNA kit (Applied Biosystems) according to the manufacturer's protocol. To demonstrate the presence of RPE in our RPE/choroid extract, we determined the expression of *RPE65* in the RPE/choroid samples by conventional RT-PCR. Samples were also tested for Rhodopsin (gene name: *RHO*), a photoreceptor marker. The primer sequences are listed in Table 3. The following PCR parameters were used: 95°C for 5 minutes, follow by 35 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 1 min, and 72°C for 5 minutes at the end of the reaction. The product was run on a 2% Agarose gel. KC (gene name: *CXCL1*) expression was determined using Taqman gene expression assays with the StepOne Plus PCR system (Applied Biosystems, Inc., Foster City, CA). The primer and probe set for KC was Mm04207460_m1, and for b-actin was Mm01205647_g1, which was used as a normalization control.

All experiments were conducted according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the research was approved by the institutional research board at Johns Hopkins Medical Institutions.

Proteins:

Full length CFH was purchased from Calbiochem or CompTech. CFH derived from genotyped AMD patients was purified from plasma as described [47]. Briefly, patient

plasma was diluted in Sterofundin (Braun, Melsungen, Germany) and applied to a HiTrap Heparin HP column (GE Healthcare, Freiburg, Germany). After loading, the column was washed extensively and bound protein was eluted using 30% Sterofundin supplemented with 1M NaCl. The eluted fractions were assayed for the presence of CFH, and the positive fractions were combined, desalted and adjusted to 25mM Tris pH 8.0. The desalted sample was applied to anion exchange chromatography using a MONO Q 4.6/100 PE column (GE Healthcare). Bound proteins were eluted with a linear gradient of 30% 25mM Tris, 1M NaCl pH 8.0 and the CFH containing elute fractions were combined, concentrated and dialyzed against DPBS (Lonza, Verviers, Belgium). Recombinantly expressed FHL1 as well as the CFH deletion constructs were prepared in the pBSV-8His baculovirus expression system as previously described [47]. In brief, *Spodoptera frugiperda* (Sf9) cells were grown in monolayers at 27°C and infected with the corresponding recombinant virus of each deletion construct using a multiplicity of infection of five. One week after infection, the culture supernatant was harvested and the recombinant contructs were purified by nickel affinity chromotography. Each protein was concentrated and dialyzed against DPBS (Lonza). Human LDL was isolated from EDTA-plasma of healthy donors after overnight fasting by differential density ultracentrifugation on OTD Combi (Sorvall) over the density range of d 1.019 to 1.063 g/ml as described [42]. The quality of LDL-preparations was checked by lipoprotein electrophoresis and protein content was measured by the Lowry method. LDL was sterile filtered and stored at 4°C. Copper sulfate–oxidized LDL (CuOx-LDL) and MDA-LDL were prepared as described previously [42]. The MAA-modifications of LDL, BSA (SigmaAldrich) or polylysine (1-4kD, SigmaAldrich) were performed as described previously [13]. Briefly, protein was adjusted to 2mg/ml and modified by reacting with 0.1M MDA in the presence of 0.2M acetaldehyde in phosphate buffered saline at pH 4.8 for 3.5h at 37°C. Unbound MDA and acetaldehyde were removed by dialysis against PBS and the degree of modification was assessed by the TNBS-test [48] as well as by the amount of specific MAA fluorescence present ($\lambda_{max.\ excitation}$ 394 nm/$\lambda_{max.\ emission}$ 462 nm). To generate 4-HNE-BSA, 2mg of BSA were modified with 5µmol 4-HNE (Alexis biochemicals) in PBS pH 9 for 24h at 37°C. Reducing conditions were maintained by adding 20mM $CNBH_3$ to the reaction. Unbound entities were removed by dialysis against PBS pH 7.4. Modification was verified by the

TNBS-assay, as well as by immunoassay using the 4-HNE reactive antibody NA59 [49]. CEP-BSA was a kind gift from Dr. John W. Crabb (Cleveland Clinic) [50]. Biotinylated proteins were generated with EZ-Link SulfoNHS-biotin (Pierce) according to manufacturer's instructions. Protein concentrations were determined by Lowry or BCA-method according to manufacturer's instructions (Pierce).

Antibodies:

MDA2 is a murine IgG monoclonal antibody [51], and EO14 a murine IgM Nab [52] that bind to malondialdehyde (MDA)-lysine epitopes present on modified LDL or other MDA-modified proteins but not to native LDL or unmodified proteins. EO6 is a murine IgM NAb that binds to the phosphocholine head group of oxidized, but not native, phospholipids as described [52]. All antibodies were purified with high performance liquid chromatography. Apolipoprotein (apo) B-100 specific monoclonal antibodies MB47 [53] and MB24 [54] (both IgG2a) against human apoB-100 were purified by protein A chromatography. Anti-human CFH antiserum and anti-mouse CFH mAb were purchased from Calbiochem and SantaCruz, respectively. In addition, human recombinant CFH (Calbiochem) was used to generate a monospecific anti-CFH antisera in guinea pigs. The IgG was purified with protein A and biotinylated according to manufacturer's instructions (Pierce) for use in assays to detect the ability of plasma CFH to bind to MDA as described below. The murine IgG2a isotype control antibody was purchased from BecktonDickinson.

Bead coupling and pulldown-procedure:

12 weeks old LDL receptor (LDLR) recombination activating gene 1 (RAG) double deficient ($LDLR^{-/-}RAG^{-/-}$) mice were set on an atherogenic diet containing 21% fat and 0.2% cholesterol (TD88137, Ssniff) for 4 weeks. After this, mice were sacrificed and heparin-plasma was obtained from the vena cava. Pooled plasma from 5 male mice was used for the pulldown experiments.

Unmodified or MDA-modified polylysine was coupled to NHS-activated sepharose (GE Healthcare) according to manufacturer's instructions. Plasmas obtained from $LDLR^{-/-}RAG^{-/-}$ mice or human donors were diluted to a concentration of 1 mg/ml total protein. To minimize the amount of proteins binding to unmodified polylsine, plasma dilutions were incubated with polylysine-coupled beads for 2h at 4°C. After the incubation, supernatant was incubated with either polylysine- or MAA-polylysine-beads for another 2h at 4°C. Beads were washed 3 times with TBS (pH 7.4, 500mM NaCl, 0.5% NP-40, 2mM $CaCl_2$, 1mM $MgCl_2$). After the last wash, bound proteins were dissociated by adding LDS-sample buffer (Invitrogen) and heating at 95°C for 5min and supernatant was used for further analysis.

Mass spectrometry:

The samples were separated by one-dimensional SDS-PAGE, digested *in situ* with trypsin and analyzed by LC-MSMS on a quadrupole time-of-flight (QTOF Premier) mass spectrometer (Waters, Manchester, UK) coupled to a 1100 series nano-HPLC system (Agilent Technologies, Palo Alto, CA). Obtained data was searched against the IPI_MOUSE database v.3.32 appended with known contaminants (e.g. trypsin, human keratin proteins).

Immunoblotting:

The samples were separated by gradient SDS-PAGE (Invitrogen) and blotted on PVDF-membranes (Whatman, Fig. 1) or nitrocellulose-membranes (Whatman, Fig. 4). Membranes were blocked in 5% non-fat dry milk in PBS with 0.05% Tween 20. Following 3 washing steps, blots were probed for the presence of CFH using goat polyclonal anti-human-CFH (Calbiochem), 1:10,000 dilution in 1% non-fat dry milk in PBS with 0.05% Tween 20 (antibody diluent) or goat polyclonal anti-mouse-CFH (SantaCruz, 1:1,000 dilution in antibody diluent). Blots with samples from the co-factor assay were probed for iC3b using goat polyclonal anti-human-C3 (Comptech), 1:2,000

dilution in antibody diluent. Anti-goat-IgG conjugated to horseradish peroxidase (Calbiochem, dilution 1:5,000 in antibody diluent) was used as a secondary antibody.

Immunoassay:

Chemiluminescent ELISA was performed as previously described [42]. Washing steps were performed on microplate washer ELx405 (BioTek). In brief, antigens at concentrations of 1-5µg/ml in PBS containing 0.27mM EDTA were added to each well of a 96-well white, round bottom microtitration plate (Thermo, MicrofluorII roundbottom) and incubated 1h at 37°C. After washing and blocking steps, the plate was incubated with purified CFH or CFH fragments at a concentration of 1-5µg/ml in TBS-BSA (TBS pH 7.4, containing 1% BSA) overnight at 4°C. Bound CFH or CFH fragments were detected with goat polyclonal anti-human-CFH (Calbiochem, 1:10,000 in TBS-BSA) and mouse anti-goat-IgG conjugated to alkaline phosphatase (AP) (SigmaAldrich, 1:30,000 in TBS-BSA). In reciprocal experiments, CFH or fragments were coated and binding of biotinylated MAA-BSA was determined. Separate experiments demonstrated the ability of polyclonal anti-human CFH to recognize all CFH fragments (data not shown). When determining potential binding of C3 (Comptech) and CRP (Calbiochem) to MAA-BSA, the purified proteins were used at a concentration of 5µg/ml and detected with either goat polyclonal anti-human C3 (Comptech, 1:4,000) or goat polyclonal anti-human CRP (SigmaAldrich, 1:10,000). For immunoassays including CRP, blocking, dilution and washing buffer contained 2mM $CaCl_2$_1mM $MgCl_2$ because many interactions of CRP, including its binding to the PC-headgroup, have been described as being dependent on bivalent cations [55].

To determine the ability of CFH in human plasma to bind to MDA-LDL or MAA-BSA, human plasma from subjects with known genotypes for the 402 polymorphism was added at varying dilutions to microtiter wells containing either coated MDA-LDL or MAA-BSA. Samples were diluted in TBS-BSA and incubated for 1 hr at room temperature. After extensive washing with a microtiter plate washer, the bound CFH was detected with biotinylated guinea pig anti-human CFH followed by AP-conjugated NeutrAvidin (PerkinElmer, 1:10,000 in TBS-BSA). AP-conjugated secondary reagents

were detected using Lumiphos (Lumigen, 50% solution in water) and a Dynex Luminometer (Dynex Technologies) and results expressed as relative light units (RLU) per 100ms.

Competition assay:

Competition assays were performed by chemiluminescent ELISA in which either binding of biotinylated MAA-BSA to coated CFH or binding of CFH (purified or in whole human plasma) to coated MAA-BSA was competed by native LDL, MDA-LDL, MAA-LDL, CuOx-LDL, BSA or MAA-BSA. Purified CFH or MAA-BSA were coated at a concentration of 2µg/ml or 0.5-1µg/ml, respectively. Biotin-labeled MDA-LDL or CFH at a concentration of 0.5µg/ml or 1µg/ml or human plasma at a dilution of 1:2,000 (in TBS-BSA) were mixed with indicated concentrations of unlabeled native LDL, MDA-LDL, MAA-LDL or CuOx-LDL and added to coated wells overnight at 4°C. Bound biotin-MDA-LDL was detected with NeutrAvidin coupled to alkaline phosphatase (PerkinElmer, 1:10,000 in TBS-BSA). Bound CFH was detected with goat polyclonal anti-human-CFH (Calbiochem, 1:10,000 in TBS-BSA) and mouse anti-goat-IgG coupled to alkaline phosphatase (SigmaAldrich, 1:30,000 in TBS-BSA). Substrate was added and luminescence was measured as described above. To express the relative binding affinities, the dissociation constants (K_dS) were determined according to the Klotz method [56]. Calculations are based on the concentration of competitor at which binding was inhibited by 50%.

Immunohistochemistry:

Cryosections were blocked with 2% normal goat serum and an avidin-biotin complex (ABC) blocking kit (Vector Laboratories, Inc., Burlingame, CA), followed by overnight incubation at 4°C with mouse MDA2 monoclonal antibody (1:2,000) or mouse IgG1, and guinea pig anti-CFH antibody (5µg/ml) or guinea pig IgG, or mouse C3d monoclonal antibody (1:100; AbD Serotec, Inc. Oxford, UK) or mouse IgG1. After

incubating for 30 min at room temperature with rat biotinylated secondary antibody (1:4,000; Vector laboratories, Inc.), followed by streptavidin APase (1:500; Sigma-Aldrich, Inc. St. Louis, MO), APase activity was visualized with a 5-bromo-4-chloro-3-indoyl phosphate (BCIP)-NBT kit (Vector Laboratories, Inc.).

Paraffin section of atherectomy samples were stained with the antibodies described above following a previously established protocol [12].

Confocal microscopy:

Co-localization of CFH and EO14 was visualized by confocal microscopy. ARPE-19 cells were incubated at 65 °C for 45 min to induce necrosis, then washed in DPBS supplemented with 1 % BSA followed by a 30 min incubation of 10 µg / ml CFH (CompTech). After a washing step monoclonal EO14 and polyclonal CFH antiserum (CompTech) were added, followed by the corresponding anti-mouse FITC (Dako, Hamburg, Germany) or anti-rabbit Alexa 647 (Invitrogen, Karlsruhe, Germany) labeled secondary antibodies. Cells were further stained with DAPI (Sigma-Aldrich) and examined with a laser scanning microscope LSM 510 META (Zeiss, Jena, Germany).

Microparticle isolation and staining:

Specific cell derived microparticles were obtained from the human T-lymphoma cell line Jurkat. After inducing apoptosis with staurosporine (1nM) for 24h, cells were sedimented and submicron particles were isolated from the supernatant by centrifugation at 16100g for 30min. The microparticles were washed three times in PBS before staining. CFH at a concentration of 10µg/ml was mixed with BSA or MAA-BSA at a concentration of 472µg/ml and incubated for 30min at 4°C. The washed microparticles were stained either with the competition mix or CFH alone for 30min at 4°C. After washing, bound CFH was detected with goat polyclonal anti-human-CFH (Calbiochem, 1:200 in PBS-BSA) and anti-goat IgG-FITC (Dako, 1:200) on a FACScalibur (BD).

Co-factor assay:

MAA-BSA at a concentration of 5µg/ml in PBS was bound to the surface of a 96-well flat-bottom microtitration plate (NUNC Maxisorp). After washing and blocking, CFH was bound to coated MAA-BSA at concentrations of 0.2-5µg/ml in PBS-BSA (PBS pH 7.4, containing 1% BSA) for 1h (if not otherwise indicated) at room temperature. If competition was performed, CFH at 5µg/ml was applied as a mixture with CFH18-20 or CFH15-19 at the indicated concentrations. Unbound protein was removed by washing and plates were incubated with C3b (Comptech, 0.8µg/ml) and factor I (Comptech, 0.2µg/ml) in PBS for 90min at 37°C. The reaction was stopped by adding LDS-sample buffer (Invitrogen) and samples were denatured at 95°C for 5min.

Macrophage Binding Assay:

Binding of biotinylated MAA-LDL to thioglycollate elicited peritoneal macrophages plated in microtiter wells was assessed by a chemiluminescent binding assay as described with modifications [42]. Briefly, isolated human LDL was biotinylated according to manufacturer's protocol (Cat# 21326; Pierce Biotechnology) prior to MAA modification. The biotinylated MAA-LDL (5 µg/ml) was incubated in the absence or presence of serially diluted CFH or BSA in 1% BSA-PBS. The ligand-competitor solutions were incubated overnight at 4°C. Thioglycollate elicited peritoneal macrophages were cultured in 10% fetal bovine serum in DMEM (DMEM-10) and plated in 100 µl L929-fibroblast conditioned media at 100,000 cells/well in sterile 96-well flat-bottom white plates (Greiner Bio-One) at 37°C. The plating media consisted of 20% L929-fibroblast conditioned DMEM-10 and 80% fresh DMEM-10 and served as a source of growth factors, including macrophage colony-stimulating factor (M-CSF). After 24 hours, plates were washed gently 5 times with PBS using a microtiter plate washer (Dynex Technologies, Chantilly, VA), and wells were blocked with 200 µl of ice-cold 1% BSA-PBS for 30 min, while plates were kept on ice. After washing, macrophages were

incubated with ice-cold ligand-competitor solutions (100 µl/well) for 2 hours on ice, washed again, and fixed with ice-cold 3.7% formaldehyde in PBS for 30 min in the dark. After fixing the macrophages, the remainder of the assay was carried out at room temperature. Macrophage-bound biotinylated MAA-LDL was detected as described above. Data were recorded as relative light units counted per 100 milliseconds (RLU/100ms) and expressed as a ratio of binding in the presence of competitor (B) divided by binding in the absence of competitor (B_0).

Stimulation of THP-1 and ARPE-19 cells:

Human THP-1 monocytic cells were cultured in RPMI-1640 supplemented with 10% FCS. The stimulation medium contained BSA or MAA-BSA at 50µg/ml and/or CFH at 200-12.5µg/ml and was incubated for 30min at RT before plating. Before stimulation, cells were washed with serum-free RPMI-1640 and incubated with the stimulation medium at a density of $5x10^5$ cells/ml for 14h. Cells were removed by centrifugation (500g, 10min) and supernatants were assayed for IL-8 by ELISA according to manufacturer's instructions (BecktonDickinson). For the simulation of THP-1 cells with LPS and phorbol-myristate-acetate (PMA), concentrations of 1µg/ml and 50ng/ml were used, respectively. ARPE-19 retinal pigment epithelial cells were seeded at $1x\ 10^6$ cells/cm^2 and grown in DMEM/F12 (1:1) plus 10% FCS until visually confluent. After serum starvation in DMEM/F12 (1:1) plus 0.1% BSA for 24 h, cells were stimulated with BSA or MAA-BSA at 50µg/ml for 24 hours in DMEM/F12 (1:1) plus 0.1% BSA. Total RNA was isolated from ARPE-19 cells using the RNeasy Mini Kit (Qiagen, Inc. Valencia, CA), and cDNA was generated by using the High Capacity RNA-to-cDNA kit (Applied Biosystems, Inc., Foster City, CA). Gene expression was measured using intron-spanning primer sets (Table 3) or on demand probe sets (Applied Biosystems, Inc.). Human IL12b quantitect primers were ordered from Qiagen. PCR reactions were analyzed by using either the Applied Biosystems 7900 HT Fast Real-time PCR system or Applied Biosystems StepOnePlus Real-time PCR system (Applied Biosystems, Inc.).

Table 3: Primers used for RT-PCR

primer designation	primer sequence	product size [bp]
hActin_fw hActin_re	cgcgagaagatgacccagatc tcaccggagtccatcacga	125
hTNFa_fw hTNFa_re	cagcctcttctccttcctga cagcttgagggtttgctaca	197
hIL-8_fw hIL-8_re	tctgcagctctgtgtgaagg acttctccacaaccctctgc	229
hIL1b_fw hIL1b_re	cagtggcaatgaggatgacttg tcggagattcgtagctggatg	117
mRPE65_fw mRPE65_re	gggaagaagttaaaagaaatgctatg ttctgcctgtgtcgacctt	96
mRho_fw mRho_re	tcacgctatcatgggtgtggtctt aggaatggtgaagtggaccacgaa	190

Stimulation of mouse bone-marrow derived macrophages:

Bone-marrow derived macrophages were generated by M-CSF differentiation of bone-marrow cells for 7 days. Purity >90% was verified by flow cytometry using an antibody directed against the macrophage-marker CD11b (BecktonDickinson) (data not shown). Cells ($1.5x10^6$ per well) were seeded in a 12-well tissue culture plate. After adherence, cells were washed 2x with PBS and stimulated with the indicated amounts of BSA or MAA-BSA in RPMI for 16h. Supernatants were cleared of cells by centrifugation (500g 10min) and were assayed for KC by ELISA according to manufacturer's instructions (R&D Systems).

Statistical analysis:

Data are presented as mean±SD or mean±SEM where indicated. Results were analyzed by one-way analysis of variance and Student's unpaired *t* test.

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SUPPLEMENTARY TABLES

Supplementary Table 1: Number of unique peptides identified exclusively in eluates from MAA-PL beads

Description	PCT PL	PCT MAA-PL
COMPLEMENT COMPONENT FACTOR H	0	19
C-REACTIVE PROTEIN PRECURSOR	0	8
BC026782 PROTEIN	0	6
ISOFORM LONG OF COMPLEMENT C3 PRECURSOR (FRAGMENT)	0	3
APOLIPOPROTEIN A-I PRECURSOR	0	3
ADULT MALE LIVER TUMOR CDNA, RIKEN FULL-LENGTH ENRICHED LIBRARY, CLONE:C730002J02 PRODUCT:FIBRINOGEN, GAMMA POLYPEPTIDE, FULL INSERT SEQUENCE	0	4
FIBRINOGEN, ALPHA POLYPEPTIDE	0	2